THE BACTERIAL EXAMINATION OF 104 SAMPLES OF WATER:

TOGETHER WITH A DETAILED STUDY OF THE COLON BACILLUS.[1]

By William G. Bissell, M.D.,
BACTERIOLOGIST, DEPARTMENT OF HEALTH, BUFFALO, N. Y.

There does not appear to be a subject at the present time that is shrouded with a greater uncertainty than the sanitary interpretation of the presence of bacilli coli in a water supply. This uncertainty has been largely augmented by the fact that many reliable observers have found colon bacilli in waters which, presumably, were not polluted with fecal material.

At the meeting of the American Public Health Association at New Orleans, La., after considerable consultation between many of the laboratory workers, it was decided that several should conduct, independently, an investigation along the following lines:

1. The consideration of what should constitute a colon bacillus.
2. The amount of water most advisable to test in order to arrive at some conservative conclusion as indicating the presence or absence of fecal pollution.
3. The length of life of the colon bacillus in water under its natural environment.
4. The degree of frequency with which colon bacilli can be found in different amounts of water known to contain sewage.
5. To ascertain, if possible, the relative degree of frequency with which colon bacilli can be detected in water presumably not polluted with sewage.

After considering the subject, it appeared to the writer that his residential district was most fortunate, in that it afforded a favorable field for an investigation of this character, and, after obtaining the co-operation of an able laboratory assistant who could devote his entire time to the work, an investigation was started.

During the progress of the work many difficulties were experienced, and particularly was this true in considering the fifth division of the work, for the reason that, although many wells were found which, upon inspection, did not reveal the probable access of fecal pollution, it was impossible to locate but one body of water (Lake Hemlock, near Rochester) where sewage pollution could presumably be eliminated.

The first division of the work, as to what constitutes a colon bacillus, was conducted along the following lines:

---

[1] Read at Washington, D. C., October 26, 1903, before the Laboratory Section of the American Public Health Association.

1. An organism responding to the full colon test was isolated from fifteen different samples of human feces. These organisms were cultured in all the common media and in different lots of the same media, and the comparative results checked. It was found that the same organism in the same class of media, but of different generations (and particularly was this true in regard to the use of the Smith fermentation tube), would frequently differ as to the amount of gas produced during a definite interval. In two instances organisms giving indol and odor reactions in older generations would fail in different lots of the same class of media in earlier generations. It was the writer's endeavor by this procedure to ascertain as near as possible the actual degree of uniformity of results that it was possible to obtain with the present colon test.

2. In ascertaining what constitutes a colon bacillus was the study of certain lactic-acid producing organisms and their comparison with known colon bacilli. Many of the members of this section will recall that Prescott has pointed out a great similarity between certain lactic-acid organisms found upon grain and the colon organism.

The writer at the meeting of the American Public Health Association at New Orleans, La., exhibited cultures showing the similarity in the cultural manifestations between the bacillus acidi lactici of Hoeppe and the colon bacillus. The work in this connection was conducted as follows:

Fifteen different laboratories were communicated with and cultures purporting to be lactic-acid bacilli were received. After thorough cultural investigation it was impossible, by any of the usual methods, to demonstrate a constant difference between ten of the cultures received and the colon bacillus. These results at first puzzled the investigators, for it suggested the possibility of the lactic-acid organism not being a distinct species of the bacteria. It is now the writer's belief that such cultures were none other than colon bacilli mistaken for the lactic-acid organism, for the reason that the remaining five cultures contained an organism absolutely non-motile; nor was it possible, under different modes of procedure, to cause these organisms to develop motility, and they in that manner differed from the colon bacillus. It is the writer's opinion that such cultures were true lactic-acid bacilli, and this has been recently strengthened by the positive demonstration of spores in four of the five cultures.

3. The next step as to what should constitute a colon bacillus was devoted to the use of presumptive tests. This work was carried on to ascertain to what degree it was necessary to carry on confirmative work in order to arrive at a positive diagnosis where a presumptive test was most marked. The organisms tested were two common to most waters, namely, the bacillus fluorescens liquefaciens and the bacillus fluorescens putridus. All results were checked with cultures of colon bacilli.

The presumptive test included the use of three fermentation tubes containing 2 per cent. of dextrose bouillon and the subsequent testing of gas formulæ after forty-eight hours, the production of acidity, the coagulation of milk, the production of indol, and the production of a disagreeable odor. It was found that the organisms mentioned gave results that could be easily mistaken for colon bacilli, if no greater time than forty-eight hours be allowed and the exact morphology of the organisms not determined.

The result of this division's work seems to justify the following conclusions:

(*a*) That the factors liable to great variation in tests for colon bacilli are those pertaining to the amount of gas produced, the production of indol, and the production of a putrefactive odor.

(*b*) That it is necessary, in order to arrive at a positive diagnosis of colon in water, to completely culture the organism, and the results obtained include the following:

1. A motile organism.
2. Slender rods without spores.
3. An acid-producing organism.
4. One that will coagulate milk within forty-eight hours without a redigestion of the casein.
5. An organism producing marked turbidity in broth within twenty-four hours.
6. An organism capable of fermenting 2 per cent. of dextrose bouillon in a Smith fermenting tube giving 25 per cent. to 75 per cent. gas production within forty-eight hours, the gas having an approximate formulæ of two parts hydrogen to one part carbon dioxide.
7. The production of indol.
8. The reduction of nitrates to nitrites.
9. The production of a putrefactive odor.
10. An organism that will ferment lactose and saccharose as well as dextrose.

(*c*) That there are other organisms common to water which may be easily mistaken for colon bacilli if presumptive tests are alone considered.

The second division of the work, as to the amount of water advisable to test in order to indicate the presence or absence of fecal pollution, was conducted as follows:

Buffalo, a city of 400,000 inhabitants, is situated at the head of the Niagara River. This river has an average velocity of nine miles per hour, and, although a rapidly running stream, is not of excessively great volume. The sewage of Buffalo is emptied into this river, and, in consequence of its being a turbulent stream, becomes mixed with the water of the river in a very short distance. It appeared to the writer that examination of different amounts of the water at distances of twelve and twenty-two miles down the stream would be productive of much valuable information as regards the amount of water advisable to test, as this river certainly received a tremendous amount of direct sewage pollution.

The investigation covered an examination of 104 samples. Fifty-two of these samples were collected twelve miles below Buffalo and the remaining fifty-two samples ten miles below this point, or twenty-two miles down stream. All samples were examined in quantities ranging from $\frac{1}{10}$ of a c.c. to 1 litre.

The results obtained were as follows: From the samples collected twelve miles down stream colon bacilli were present in:

21	per cent. when the amount examined was	1/10 c.c.
29	" " " " "	½ "
52	" " " " "	1 "
59	" " " " "	5 "
69	" " " " "	10 "
80	" " " " "	50 "
87	" " " " "	100 "
90	" " " " "	500 "
91	" " " " "	1 litre.

Of the samples collected twenty-two miles down stream the results were as follows:

19	per cent. when the amount examined was	1/10 c.c.
21	" " " " "	½ "
34	" " " " "	1 "
39	" " " " "	5 "
41	" " " " "	10 "
47	" " " " "	50 "
51	" " " " "	100 "
53	" " " " "	500 "
69	" " " " "	1 litre.

There were several doubtful results during the progress of the work, and all such results were recorded as being positive.

In several instances, if the presumptive test mentioned in Division No. 1 was alone considered as being conclusive evidence of the presence of colon bacilli, many errors would have resulted, and a much larger percentage of positive results recorded. As it is, the result of this work does not correspond with similar work conducted by Winslow and Miss Hunnewell, as recorded in a statement issued by Prescott, in that the percentage of 1 c.c. of samples examined revealing positive evidence of colon bacilli are much larger than in their investigation. This may be accounted for by the fact that it is the writer's belief that the relative frequency of colon bacilli in a given quantity of water is largely dependent upon the amount of dilution that the raw sewage or fecal material undergoes. It will be noted that the results at twelve and twenty-two miles show a considerable variance in favor of colon bacilli being less frequently found in the same amounts of water at the greater distance. The cause of this decrease does not seem easy of explanation, for the reason that the natural influences which tend to produce disintegration of organisms as worked out and recorded by Jordan do not seem to be present in this clear, shallow, rapidly running stream, having a bed largely composed of rock.

Agitation would not seem accountable for the result, for the reason that samples collected immediately above and below Niagara Falls failed to reveal any difference in the number of organisms present. It would seem to the writer that sunlight is the only remaining natural factor that could possibly be considered, and the reduction may be due to this cause. The land on either side of the Niagara River is subject to constant cultivation, and, as many of these samples were taken during and following heavy rainfalls, it would seem, if the cultivation of lands in the near vicinity supplies any great amount of colon bacilli to a stream of this character, that the relative numbers of coli would have been materially increased at such times. As a matter of fact, this was not the investigator's experience. The number of coli present in the amount of water was materially lessened following rainfalls. This, of course, might be due to the great dilution produced by the rainfall. As judged by the investigators, the relative amount of colon supplied, if any, must have been extremely small to permit of the reductions noted. As a recapitulation of the results of the second division's work, the following conclusions would seem justifiable:

1. The presence of colon bacilli in the majority of 10 c.c. lots of a sample should be looked upon as suspicious.
2. When found in the majority of 5 c.c. lots of a sample it should be looked upon as decidedly suspicious.
3. When found in 50 per cent. of 1 c.c. lots of a sample it can be considered as evidence of direct fecal pollution.
4. That whereas cultivated lands bordering on water supplies may furnish sufficient quantities of colon bacilli during certain periods to permit of their being detected when large quantities of water are examined, it does not appear that in the examination of 1 c.c. amounts of samples that colon bacilli coming from this source will be present in sufficient numbers to permit of their detection in the majority of the 1 c.c. examinations.

The third division of the work, the length of life of the colon bacillus in water under natural environment, was conducted as follows:

Three wells were found, which, after thorough inspection of the surrounding territory and careful bacterial examination, did not reveal colon bacilli. There was placed in each well one quart of excrement, the source of same being as follows:

Human in Well A, cow in Well B, and horse in Well C.

After ten days all wells were examined and colon bacilli were found in abundance, the exact number being in the neighborhood of: Well A, 500 to 800 per c.c.; Well B, over 3000 per c.c.; Well C, over 1000 per c.c.

After thirty days the number of colon in Well A were approximately the same; in Well B the colon had greatly decreased; in Well C the colon had decreased 10 per cent. After ninety days the number of colon bacilli in Well A had decreased over 11 per cent.; in Well B could be found only occasionally in c.c. quantities, and in Well C there was but an average of four colonies of colon bacilli to the c.c., counting four plates. It was impossible at this time to continue the investigation of Wells B and C, but an examination of Well A one hundred and ninety days after the introduction of excrement revealed five colonies of colon bacilli per c.c., averaging three lactose litmus agar plate. From these tests it would seem that the length of life of colon bacillus in well water under natural conditions varies with the variety. It was impossible to conduct an investigation relative to the length of life of colon bacilli in stream water. The following recapitulation of results would seem justifiable:

1. The resistance of the colon bacillus in water is influenced by the animal source of the material.
2. That there is a gradual decrease in the number of organisms where there is no additional pollution.
3. That colon bacilli in human excrement is capable of surviving at least one hundred and ninety days in well water.

The fourth division of the work, that of considering the degree of frequency with which colon bacilli can be found in water known to be polluted with sewage, is covered under the results reported in the work of the second division.

The fifth division of the work, the degree of frequency with which colon bacilli can be found in waters not presumably contaminated with fecal material, was conducted as follows:

The work of this division was mostly confined to the examination of well waters, for the reason that after a most diligent search the investigators were unable to locate in the near vicinity to Buffalo a single stream, and but one lake (Hemlock, near Rochester) from which the presence of sewage could be presumably eliminated. The source of this lake is said to be springs, and it is not immediately connected with any other great body of water. The great distance of this lake from the point where the work was conducted rendered it impossible to obtain any great number of samples for investigation. Seven samples were examined, all being collected upon the same day, at different locations in the lake, and, fortunately, at a time following a forty-eight-hour rainfall. The land surrounding Lake Hemlock for a considerable number of feet is controlled by the city of Rochester, and is protected as a water-shed to this lake. The remaining adjacent lands are used for farming purposes.

After many attempts, using 1 c.c., 3 c.c., 5 c.c., and 10 c.c. lots from each of the seven samples, it was impossible to detect the presence of colon bacilli. The well-water tests included, on an average, four examinations of two different samples from thirty-one different wells. 5 c.c. was the amount selected in each instance. None of the examinations revealed the presence of colon bacilli.

A recapitulation of the results of the five divisions of the work seems to justify the following conclusions:

1. That there is a difference between the bacillus acidi lactici of Hoeppe and the colon bacillus.
2. That presumptive tests, although of unquestionable value as primary procedures, and serviceable for prolonged routine test of a supply, where the analyst is familiar with all conditions and can ascertain at a glance any variable feature, must not be relied upon in arriving at a positive definite diagnosis of the colon bacillus.
3. That the colon bacillus cannot be constantly detected in c.c. amounts of water from the same source, although it may be heavily polluted with sewage.
4. That the presence of colon bacilli in 50 per cent. of c.c. amounts of water is strongly indicative of sewage pollution.
5. That the ordinary cultivation of farm lands, although it may be the source of some colon bacilli to a water supply, does not seem to play any great role as such, and that this source need hardly be considered when the majority of 1 c.c. samples reveal the organism.
6. That agitation has little or no destructive influence upon colon bacilli in water, and that light appears to exert a destructive influence.
7. That the positive detection of the presence of true colon bacilli in waters not presumably receiving sewage is rare.
8. That it is possible there have been organisms mistaken for colon bacilli, probably through too great reliance upon presumptive tests.

OBSERVATIONS UPON THE CHROMATIC VARIATIONS IN THE PRECIPITATED AND SEDIMENTED CHLORIDES, SULPHATES, AND PHOSPHATES OF URINE.[1]

By Edward F. Wells, M.D.,

AND

John C. Warbrick, M.D.,
OF CHICAGO.

In making quantitative analyses of urine for chlorides, sulphates, and phosphates by the volumetric method, using the centrifuge for the purpose of throwing down the precipitate, certain chromatic variations in the sediment have long attracted our attention. Although

---

[1] Read before the Chicago Medical Society, January 6, 1904.